Expression of Laminin in Hepatocellular Carcinoma: An Adjunct for Its Histological Diagnosis

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In general, hepatocytes lack basement membrane structures and therefore no laminin expression is seen around hepatic cords. To determine whether or not laminin expression appears when hepatic tissue becomes carcinomatous, we carried out immunohistochemical staining of hepatic tissues excised surgically from 35 patients with hepatocellular carcinoma, 18 with metastatic colon carcinoma, two with adenomatous hyperplasia, and 10 without any nodular lesions. Among the various conditions of hepatic tissue, laminin expression was detected only in hepatocellular carcinoma with 86% positivity. The result was not dependent on the degree of differentiation. Therefore, it was confirmed that immunohistochemical detection of laminin provides a useful adjunct for the diagnosis of hepatocellular carcinoma, and this was verified by a study using needle biopsy samples. In addition, our results suggested that the basement membranes are derived from endothelial cells of either portal veins or hepatic arteries.

Introduction

A well differentiated hepatocellular carcinoma (HCC) may histologically resemble normal and/or regenerative liver tissue,[1–3] and it is often difficult to differentiate from the latter conditions, especially in needle biopsy specimens.

Laminin is a component of the extracellular matrix and exists within the basement membrane.[4, 5] In normal liver tissue, well defined basement membranes never surround the hepatic cords, and therefore laminin is not present along the sinusoids.[6, 7] In HCCs, however, laminin may be expressed around tumor cell nests, and is detectable by immunohistochemistry.[8–10] If, indeed, laminin appears only in carcinomatous liver tissue, its detection could be a useful adjunct for histological diagnosis. To verify this possibility, we carried out immunohistochemical staining of laminin in tissues of HCC, liver cirrhosis, and chronic hepatitis as well as non-neoplastic, non-inflammatory liver. Our results suggested that laminin is expressed only in

carcinomatous liver tissue and that it is a useful marker for diagnosis of HCC.

Materials and Methods

For this study, we used hepatic tissues excised surgically from 35 patients with HCC, 18 with metastatic colon cancer in the liver, two with adenomatous hyperplasia and 10 without any nodular lesions (Table I). Then, to determine whether or not the same principle could be applied to needle liver biopsy specimens, and whether detection of laminin would be useful for the diagnosis of HCC, 100 biopsy samples, including 29 samples of HCC, 23 of liver cirrhosis, 41 of chronic hepatitis, and seven of normal liver were utilized (Table II). The diagnosis in each case depended upon not only histological changes but also preoperative biochemical data, the serum levels of tumor markers, and the results of diagnostic imaging, including computed tomographic scans of the liver and echography. For the first study, HCCs were histologically subclassified into well differentiated, moderately differentiated, poorly differentiated and undifferentiated carcinomas according to the classification of The General Rules for the Clinical and Pathological Study of Primary Liver Cancer.[11] Because a single surgically excised nodule of HCC may com-

Table I. Diagnosis of the Resected Samples

Nodular lesions		Diagnosis of nodules		Diagnosis of surrounding tissue		
				normal	CH	LC
(+)	55	HCC	35	0	9	26
		Meta.	18	12	4	2
		AH	2	0	0	2
(−)	10			0	4	6
Total	65		55	12	17	36

HCC, hepatocellular carcinoma; Meta., liver metastasis from colon carcinoma; AH, adenomatous hyperplasia; CH, chronic hepatitis; LC, liver cirrhosis.

Table II. Diagnosis and Expression of Laminin in Needle Samples

Laminin expression	normal	CH	LC	HCC
positive/total	0/7	0/41	0/23	18/29
%	0	0	0	65.5

*, $P < 0.025$ (between normal and HCC); †, $P < 0.005$ (between CH and HCC, LC and HCC); CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

Table III. Laminin Expression in Resected Samples

Laminin	normal	CH	LC	AH	HCC (degree of differentiation)				
					all of HCC	well	mod.	por.	un.
positive/total	0/12	0/17	0/36	0/2	44/50	23/26	17/17	3/5	1/2
%	0	0	0	0	88.0	88.5	100	60	50

*, $P < 0.005$ (vs HCC); †, $P < 0.025$ (vs HCC); CH, chronic hepatitis; LC, liver cirrhosis; AH, adenomatous hyperplasia; HCC, hepatocellular carcinoma; well, well differentiated; mod., moderately differentiated; por., poorly differentiated; un., undifferentiated.

prise areas which differ in their degree of differentiation, we divided the lesions into areas showing specific degrees of differentiation and counted each area as a single lesion. This gave a total of 26 well differentiated, 17 moderately differentiated, five poorly differentiated and two undifferentiated lesions. We utilized non-tumorous liver tissue samples as controls. Because the liver tissue adjacent to HCC contained cirrhosis in 26 cases and chronic hepatitis in nine, and that adjacent to metastatic colon cancer was normal in 12 cases, showed chronic hepatitis in 4 and cirrhosis in two, the control materials finally included 36 samples of cirrhosis, two of adenomatous hyperplasia, 17 of chronic hepatitis and 12 of normal liver.

All the tissues were fixed in 10% buffered formalin, processed routinely and embedded in paraffin. Four-micrometer-thick sections were obtained for histological examination and stained with hematoxylin-eosin, and some of them were stained immunohistochemically with an anti-laminin antibody. For immunohistochemistry, we used the avidin-biotin-peroxidase complex (ABC) method.[12, 13] Briefly, the sections were deparaffinized and incubated with 0.1% pepsin (Sigma P-6887, Sigma Chemical Co., St. Louis, MO)/0.01 N-HCl at 37°C for 100 min. After washing, they were again incubated with 0.05% pronase (Sigma P-6911) in phosphate-buffered saline at 20°C for 10 min.[14] Intrinsic peroxidase activity was inhibited by 0.3% H_2O_2-methanol, and any non-specific reaction was blocked with normal goat serum. Next, the tissue sections were reacted with a rabbit anti-human laminin antibody (polyclonal; Bio-science Products AG, Emmenbrücke, Switzerland) for 120 min at 20°C and then with an Elite ABC kit (Vector Laboratories Inc., Burlingame, CA). Finally, 3,3'-diaminobenzidine tetrahydrochloride was used as a chromogen, and the sections were counterstained with Carazzi's hematoxylin, dehydrated, and mounted. Dark brown linear deposits along and/or around the hepatic cords were considered to indicate positive result. Positive and substitution controls were stained in parallel with the test materials. The positivity of laminin expression in normal, inflammatory and neoplastic liver tissue was compared and tested by χ^2 analysis.
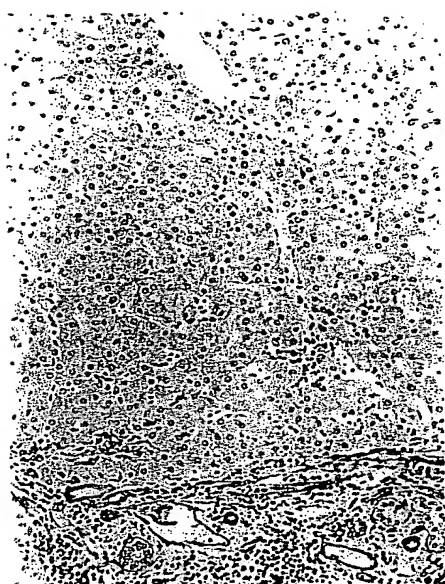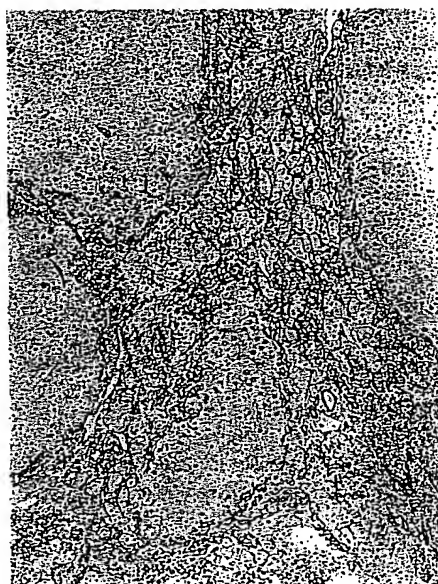
Fig. 1. Immunohistochemistry of laminin in chronic hepatitis. Immunoreactivity is absent in the perisinusoidal space, although there is positive expression of laminin in the basement membrane of vessels and biliary ducts in the portal area.
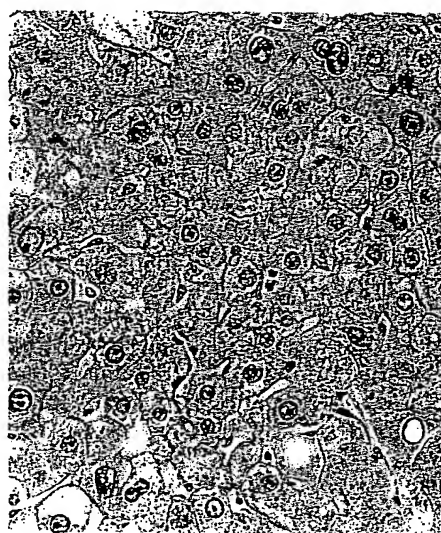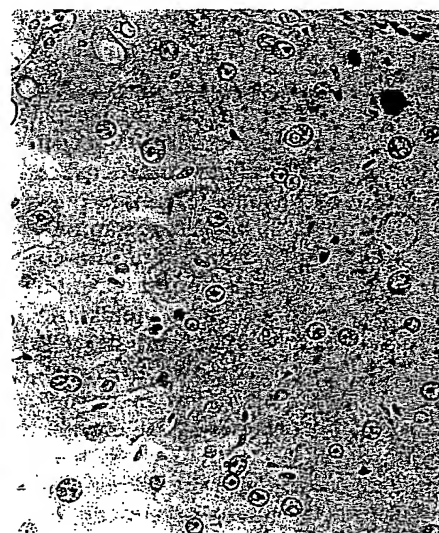

(a)   (b)

Fig. 2. Immunohistochemistry of laminin in cirrhotic liver tissue. A linear reaction is seen at the periphery of the pseudolobules. Note that the hepatocytes within the pseudolobules are not stained for laminin.

(a)   (b)

Fig. 3. Liver cirrhosis. Nuclear atypism is prominent in some lesions, but laminin reactivity is absent (3a: HE, 3b: ABC).
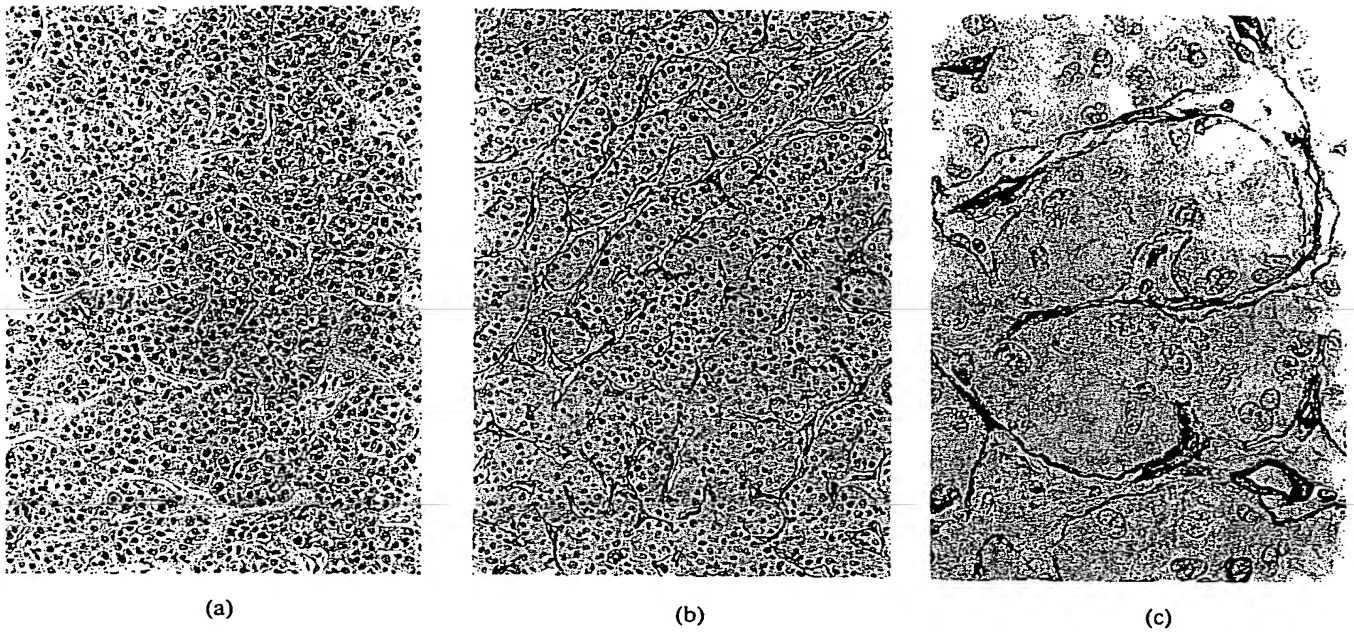
(a)　　　　　　　　　　(b)　　　　　　　　　　(c)

Fig. 4.　Immunohistochemistry of laminin in HCC. A linear positive reaction is seen in the perisinusoidal space, surrounding a nest of HCC (4a: HE, 4b-c: ABC).
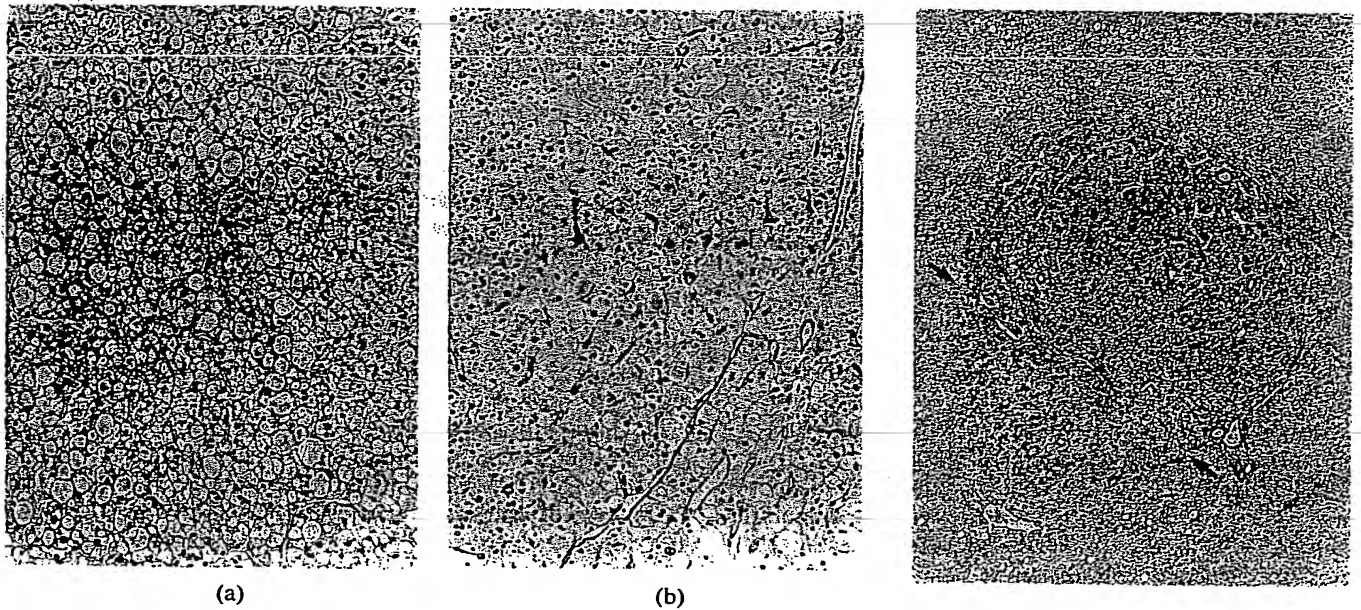

(a)　　　　　　　　　　(b)

Fig. 5.　Well differentiated hepatocellular carcinoma. Fatty change and little nuclear atypism would hinder the recognition of this tumor as hepatocellular carcinoma. However, because laminin expression is evident along the sinusoids in places, the tumor can be positively diagnosed (5a: HE, 5b: ABC)

Fig. 6.　Immunohistochemistry of laminin in a small HCC nodule. Immunopositive materials are seen near the portal area (arrows) but not in the central.
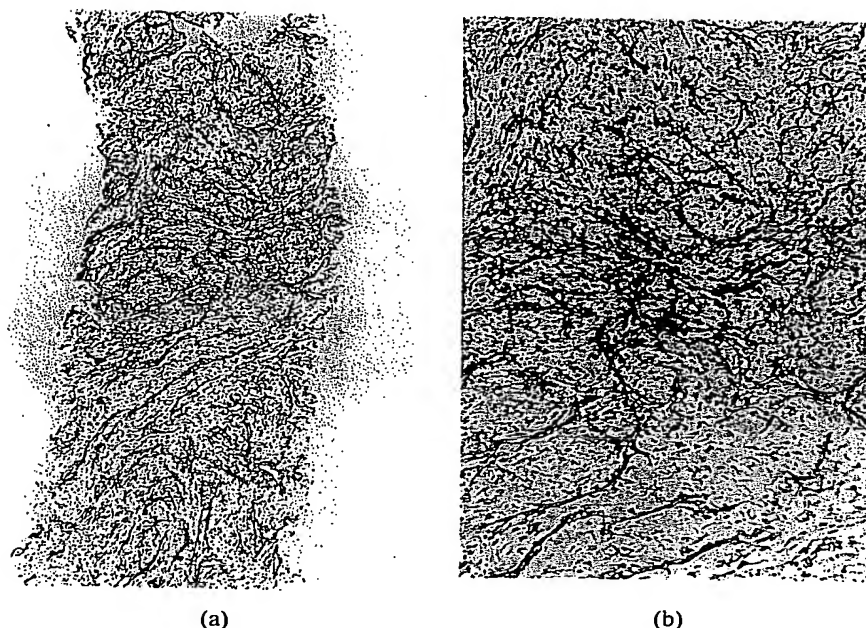
(a) (b)

Fig. 7. Immunohistochemistry of laminin in a biopsy sample of HCC. The pattern of immunoreaction is similar to that in resected samples. The cells are poorly differentiated, spindly carcinoma cells.

Results

Among the liver tissues taken by excision, laminin reactivity was detected only in HCCs (Table III). In normal and chronically inflamed tissues, no laminin expression was seen within the hepatic lobules (Fig. 1). Cholangioles and blood vessels in the portal areas, however, were surrounded by linear immunoreactive deposits, as were colonic adenocarcinomas. Cirrhotic liver showed a distribution pattern essentially similar to the normal one, except for a few cases where linear reaction products were present at the extreme periphery of the pseudolobules, surrounding hepatocytes contiguous with the portal venous or arterial system (Fig. 2a, b). In the latter cases, cholangioles merging into hepatic cords were also immunopositive. However, the remaining hepatocytes in the pseudolobules were consistently negative (Fig. 3a, b). Areas of adenomatous hyperplasia were also negative for laminin. In contrast, laminin was positive in 30 out of 35 cases (86%) of HCC. Generally, linear laminin-positive deposits were located between the sinusoidal endothelium and hepatoma cells, surrounding nests of carcinomatous hepatocytes (Fig. 4a, b, c). When laminin was stained, it was distributed evenly within the tumor mass, irrespective of the location. When the degree of differentiation was taken into account, 23

of 26 well differentiated (Fig. 5a, b), 17 of 17 moderately differentiated, 3 of 5 poorly differentiated, and 1 of 2 undifferentiated lesions were positive for laminin (Table III). There were no significant differences in positivity among carcinomas with different degrees of differentiation or architectural patterns ($P > 0.05$). On the other hand, in some tumors, particularly small ones less than 1 mm in diameter, positive reactions were seen near portal areas but not in the central portion (Fig. 6). In addition, large trabeculae of HCC exhibited widely separated immunopositive basement membranes.

In biopsy samples, the results were similar to those for resected materials with regard to positivity (Table II) and distribution pattern (Fig. 7a, b). Overall immunopositivity in HCCs was 62%. All the other tissues were completely negative for laminin expression.

Statistical analysis indicated a significant difference in immunopositivity between benign hepatic tissues and hepatocellular carcinoma (Tables II and III).

Discussion

It is generally said that the basement membrane does not exist in normal hepatic lobules. This was

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first noted in electron microscopy studies, which found that electron-dense basement membrane materials were absent between hepatocytes and sinusoidal endothelial cells, [6, 15-17] and then in immunohistochemical studies where hepatic cords were found to lack surrounding laminin,[7, 10] despite the presence of type IV collagen, another component of the basement membrane. This unique structure, as well as fenestration of endothelial cells, is considered to facilitate the transport of a variety of substances between the blood and hepatocytes. However, it has been demonstrated ultrastructurally that electron-dense materials of the basement membrane may appear in association with liver fibrosis.[7, 10] Interestingly, immunohistochemical expression of laminin never appears in the central portion of pseudolobules,[7, 10] but at their extreme periphery. In HCCs, laminin expression develops beneath the endothelial cells surrounding nests of carcinoma cells,[8, 18] as shown in this study.

There are two implications of the present data: (1) laminin expression appears in HCC and (2) laminin expression or the formation of basement membrane may be carried out by endothelial cells of the portal venous or arterial system. In our study with resected materials, the presence of laminin immunoreactivity was detected only in cases of HCC, implying that laminin expression in hepatocytic lesions was specific for HCCs. This feature may be important in surgical pathology because the immunohistochemical presence of laminin might be an excellent marker for diagnosis of HCC in cases where it is histologically difficult to differentiate from regenerated hepatocytes, particularly in small needle biopsy samples. However, not all HCCs expressed laminin, indicating that there is a limitation to its usefulness. Therefore, we undertook a similar study using needle biopsy samples to determine how accurately the results could be used as an indication of malignancy. In contrast to the overall positivity of 86% in resected samples, that of the needle biopsy samples was 62%. This difference between the two procedures may be due to the amount of tissue taken in each. In HCCs with thin trabecular structures, laminin deposits were located close to each other, but those of thick trabecular structures were rather widely separated. In other words, the amount of laminin may vary according to the thickness of the hepatoma trabeculae, and in thicker trabeculae the positivity becomes less than that in thin ones. This is because in carcinomas with thick trabecular structures, the needle tract may not pass through many portions where laminin is located. Absence of laminin in the central portion of a minute hepatoma does not hinder the usefulness of this procedure for detection of HCC, because a 1-mm hepatoma nodule would not be

detected clinically or biopsied intentionally. Another feature necessitating caution is the presence of laminin exprerssion at the extreme periphery of pseudolobules in cirrhotic liver, and so possible over-reading should be avoided. However, bearing these possible pitfalls in mind, we conclude that detection of laminin in the diagnosis of HCC is useful.

The origin of laminin in HCC still remains to be elucidated. The expression of laminin may result from sinusoidal capillarization in hepatocellular carcinoma. However, our study shows there are several reasons to believe that laminin may be produced by endothelial cells of either the portal veins or hepatic arteries, although it cannot be completely ruled out that carcinomatous hepatocytes can produce these laminins. First, laminin was expressed in the outermost portions of pseudolobules in cirrhotic liver, which are connected to blood vessels of portal areas, particularly portal veins. Second, HCC is believed to be nourished by hepatic arteries and portal veins.[9, 19-21] Third, small nodules of HCC in our study showed an uneven distribution of laminin, the expression being stronger in zones near portal areas and absent near the central vein. From the above findings we can speculate as follows. HCC cells may show active proliferation, but they may not necessarily have the same functions as normal hepatocytes. Therefore they may require more nutrients and oxygen, which may be supplied by either the portal veins or hepatic arteries. For this reason, as the tumor grows, endothelial cells from these vessels must extend or replace sinusoidal structures with basement membrane material. To validate this hypothesis, further studies will, of course, be necessary.

In summary, our study has clearly demonstrated that laminin expression is present only in carcinomatous liver tissue and is useful for distinguishing HCC from normal or regenerative hepatic tissue. Therefore, this feature is expected to be a useful adjunct for histological diagnosis in difficult cases.

Acknowledgments

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